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# Plant protein digestibility

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Better understanding of plant-based proteins digestibility can improve their utilization in human nutrition. The objective of this study was to review the current literature for digestibility of plant-based proteins. A total of 116 articles were identified from PubMed with full-text accessibility through Primo. In this review, in vitro, animal, and human studies were eligible for inclusion.

In vitro methods consisted of static and semi-dynamic methods and the INFOGEST protocol. Animal studies used rats and pigs to measure digestibility, with fecal nitrogen loss or T-cannula being the most common methods used. In humans, labeled protein was commonly used. In the studies, tested proteins were often processed via protein isolates, extrusion, or heating.

The experimental methods and protein processing had a great impact on the results, suggesting an elimination of factors present in plant-based sources that reduce digestibility, such as cell wall and antinutritional factors. The digestibility of plant-based proteins was reported to be in the range of 63–100 %, showing the capability of gastrointestinal enzymes to digest plant-based proteins. In conclusion, plant-based sources can be considered suitable protein sources for human nutrition.

## Abbreviations

| | |
|---|---|
| AA | Amino acid |
| ABP | Animal-based protein |
| DH | Degree of hydrolysis |
| GI | Gastrointestinal |
| IAA | Indispensable amino acid |
| PBP | Plant-based protein |
| pI | Isoelectric point |
| SID | Standardized ileal digestibility |
| TID | True ileal digestibility |
| TPD | True protein digestibility |

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# 1 Introduction

The world's population is growing, and it sets challenges for food production. Protein is necessary for humans and protein deficiency is already a problem in some parts of the world, for example most parts of Africa and South-East Asia has prevalence of stunted children over 32 % (Ghosh et al. 2012) even though since 1961 total meat production has more than quadrupled (Ritchie 2017).

Increased consumption of animal-based proteins (ABP) is a challenge for the environment for example due increased deforestation, agricultural pollution and greenhouse gases from livestock (Espinosa-Marrón et al. 2022). Thus, there is a demand for more plant-based food consumption. Plant-based protein (PBP) sources can provide more sustainable options for the increasing protein need of the growing population. However, amino acid (AA) composition in PBP may not be optimal for human nutrition since they often fall short in one or more indispensable amino acids (IAA) (Pinckaers et al. 2021), although some PBPs, i.e., potato has been shown to meet the IAA requirements recommended by WHO/FAO (Gorissen et al. 2018). In addition, the structure or components of the plant matrix may impede the factors interfering bioavailability of proteins in PBPs (Kårlund et al. 2021).

Plant cell structure includes factors that affect digestibility, such as cell wall, antinutritional factors and surface charge of the proteins, and these have an impact on digestibility (Carbonaro et al. 2015, Santos-Hernández et al. 2020). Processing has been shown to improve digestibility by eliminating or decreasing the amount of some of these factors (Rutherfurd et al. 2015, Nosworthy et al. 2017a). Some commonly used processing methods for example are fermentation, extrusion, chemical extraction (e.g., alkaline), enzymatic treatment, precipitation, cooking, and mincing (Mäkinen et al. 2017, Brishti et al. 2020, Emkani et al. 2021, Kumar et al. 2021).

Digestibility can be assessed either in vitro or in vivo and both methods are commonly used in studies evaluating digestibility of PBP. In vitro methods are usually used to measure soluble

nitrogen but there can be different settings used, such as use of static or dynamic settings. Rats and pigs are recommended animals to use in in vivo studies (de Vrese et al. 2000, Food and Agriculture Organization of the United Nations 2013). In addition to these research methods, human studies provide supporting evidence for digestibility of PBPs (Gaudichon et al. 2002).

The aim of this review is to summarize research investigating the digestibility of PBPs. A better understanding of PBP digestibility is needed for improving overall nutrition and PBP utilization. The focus will be on studies done in vitro and in vivo with animals and humans on digestibility of common PBP sources.

# 2 Methods

The literature review was done by using PubMed for the article search. Full articles were then searched from University of Eastern Finland internet library, Primo. Search terms and results are given in **Table 1**. Search results were reviewed based on abstract, and 59 articles were chosen for full text review. After full text review, 43 articles were chosen to be used in this review.

**Table 1.** PubMed search terms used for the review

| Title/Abstract | AND | OR | Results | Starting from (year) |
|---|---|---|---|---|
| plant protein | digestion | utilization | **162** | |
| soy protein | utilization | digesti* | **434** | **2008** |
| pea protein | utilization | digesti* | **85** | |
| ("Digestion"[Mesh]) AND "Plant Proteins, Dietary"[Mesh] AND utilization[title/abstract]) | | | **8** | **2010** |
| ("Digestion"[Mesh]) AND "Plant Proteins, Dietary"[Mesh] AND digest*[title/abstract]) | | | **165** | **2010** |

References from the originally identified articles were reviewed and an additional 57 other articles were identified, of which 37 were deemed relevant.

# 3 Plant proteins in nutrition

## 3.1 Proteins in plants

PBP sources have structural factors that can affect digestibility (Santos-Hernández et al. 2020). First is the outer layer of protein source, e.g., cell wall that enzymes in human gastrointestinal (GI) tract are only partly or not at all hydrolyzing. Therefore, the cell wall is a major factor that reduces accessibility to proteins and may reduce protein digestibility (Santos-Hernández et al. 2020). The second layer is endosperm and the third is protein bodies (Duodu et al. 2003).

**Table 2.** Storage proteins in plant-based sources

| Authors | Source | Major storage proteins | Amount (%) |
|---|---|---|---|
| Song et al. 2016 (India), Yang et al. 2016 (China) | Soy | Globulin | 70-80 |
| | | Other | <10 |
| | | Lectin + KTI | <10 |
| Acquah et al. 2020, Lu et al. 2020 (Canada) | Pea | Globulin | 55–80 |
| | | Albumin | 10–25 |
| | | Prolamin | 4–5 |
| | | Glutelin | 3–4 |
| Alghamd 2009 (Saudi Arabia) | Faba bean | Globulin | 69.5–78.1 |
| | | Glutelin | 12.0–18.4 |
| | | Prolamin | 1.83–3.57 |
| | | Albumin | 1.41–3.01 |
| Mendoza et al. 2001 (Japan) | Mung bean | Globulin | 89 |
| | | Vicilin | 3.4 |
| | | Glycinin | 7.6 |
| Amagliani et al. 2017 (Ireland) | Rice | Glutelin | 75–81 |
| | | Globulin | 7–17 |
| | | Albumin | 5–10 |
| | | Prolamin | 3–6 |
| Dizlek and Awika 2022 (USA) | Wheat | Gliadin | 31.9–54.3 |
| | | Glutenin | 29.8–42.9 |
| Rasane et al. 2015 (India) | Oat | Globulin | 80 |
| | | Prolamin | 15 |
| | | Glutelin | 5–66 |
| | | Albumin | 1–12 |

Proteins are stored in structured form and the most common molecular structures in plants are globulin, albumin, prolamin, glycinin, and glutelin (Mendoza et al. 2001, Fujiwara et al. 2002, Santos-Hernández et al. 2020). **Table 2** lists examples of common PBP sources and the main

protein storage forms. Commonly glutelin content of <10 % is reported in literature (Mäkinen et al. 2017). However, Rasane et al. (2015) concluded that glutelin content in oat is 5-66 % depending on the used extraction method. In addition, different cultivars may provide different contents (Alghamd 2009).

### 3.1.1 Protein storage

Albumin is resistant to proteolysis by trypsin, chymotrypsin, and pepsin or by heat treatment (Pantoja-Uceda et al. 2004, Carbonaro et al. 2015). It is due to the high amount of cysteine residues that can form intra chain disulfide linkages, thus increasing stability via the compact structure and self-association. The stability of these small molecules enables them to cross gut mucosal and sensitize the immune system (Carbonaro et al. 2015). Similar mechanism has been shown in wheat where high proline and glutamine content makes proteins resistant to GI enzymes leading to longer oligopeptides remaining in small intestine (Perez-Gregorio et al. 2018).

Globulins are abundant in legumes, and they can be divided into two subgroups: legumin and vicilin (Martineau-Côté et al. 2022). Legumins have lower solubility due to the β-subunit content than vicilin which consist of α- and α'-subunits. This is reflected in the thermal stability with 65.7–80.8 °C and 93.5–95.2 °C for vicilin and legumin, respectively, depending on the protein source (Kimura et al. 2008). Globulins in soy mostly consist of glycinin and β-conglycinin (70–80 %), these impair the bioavailability of soy proteins (Mariotti et al. 1999, Yang et al. 2016, Song et al. 2022).

## 3.2 Plant protein digestibility

### 3.2.1 Definition of digestibility

Assad-Bustillos et al. (2020) defined digestibility as: "Digestibility is defined as quantity of a compound released from its matrix and becoming available for absorption in the stomach and GI tract". Apparent ileal digestibility of proteins is defined as disappearance of ingested dietary

AA from the digestive tract before reaching distal ileum, and if corrected for the basal endogenous losses of AAs, it is described as standardized ileal digestibility, SID (Stein et al. 2007).

The difference between SID and true ileal digestibility (TID) is that TID takes into account the total endogenous AA losses (Stein et al. 2007). Endogenous losses are AAs that are secreted, including bacterial proteins, during digestion and that are not reabsorbed before reaching the distal ileum (Stein et al. 2007). Fecal nitrogen loss is used to estimate digestibility by subtracting the measured nitrogen in feces from total nitrogen intake (Brishti et al. 2020). Complete calculation is shown in **Equation 1** and a slightly modified formula is often used to calculate SID, TID and TPD.

$$D\,\% = \frac{I - (F - F_r)}{I}$$

**Equation 1.** Digestibility calculation for fecal N loss. D=digestibility, I= intake, F=fecal nitrogen, $F_r$=fecal nitrogen with protein free diet.

### 3.2.2 Isoelectric point or Protein charge

Proteins have the lowest digestibility around their isoelectric point (pI) where the AAs effective net charge is close to zero at a specific pH (Mota da Silva et al. 2021). This pI is usually between pH three to six for PBPs, though for example for wheat the pI range is 3.5–8 (Pearsall and Ewing 1924, Carbonaro et al. 2015, Brishti et al. 2020). **Figure 1** shows the pI for soy and pea. Solubilization of protein is enhanced by electrostatic repulsion and hydration when a protein carries a positive or negative net charge. This is shown in **Figure 2** with mung bean where solubilization is lowest between pH 4–6 which corresponds to the isoelectric region of mung bean. The susceptibility of a protein to proteolysis is reduced by its hydrophobicity, while the solubility is improved by an increase of interactive surface region between water and protein molecule (Carbonaro et al. 2015, Brishti et al. 2020).

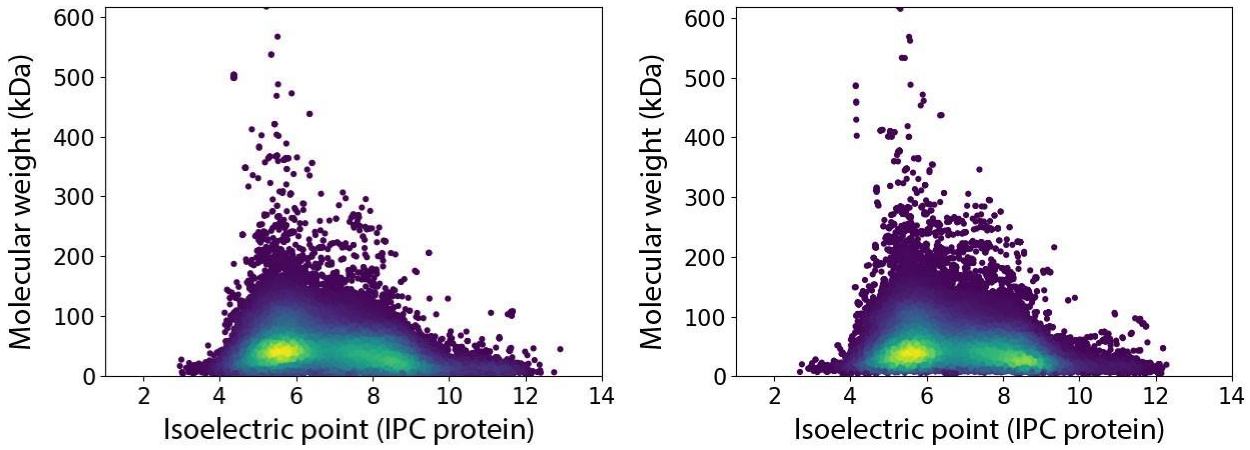


**Figure 1.** 2D Map of molecular weight and isoelectric point for pea (Vinga unguiculata) (left) and soy (Glycine max) (right). (Mohanta et al. 2022)

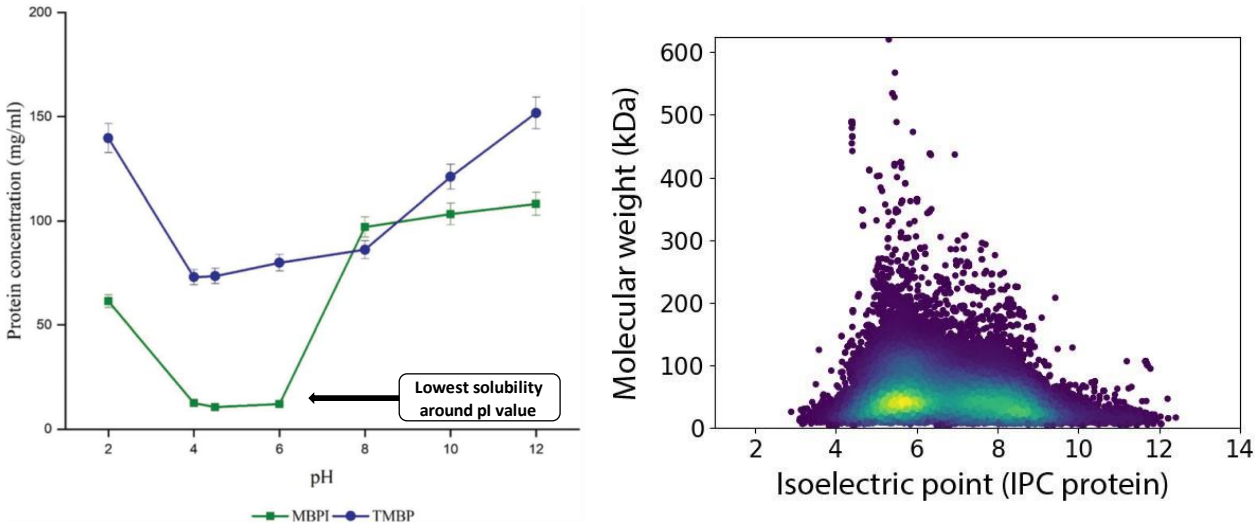


**Figure 2.** pI value and effect to protein solubility of mung bean protein isolate (MBPI) and texturized mung bean protein (TMPI) (left) and 2D Map of molecular weight and isoelectric for mung bean (Vigna radiata) (right). Modified from Brishti et al. (2020). (Mohanta et al. 2022).

### 3.2.3 Antinutrients

PBP sources contain factors that reduce digestibility of proteins either by inhibiting the digestive enzymes or forming complexes by interacting with proteins (Kårlund et al. 2021). These are generally called antinutritional factors. There are different protease inhibitors which inhibit the action of digestive enzymes (Kårlund et al. 2021). These can be indicated as larger particle sizes in vitro after gastric phase due to their resistance to pepsin hydrolysis (Jiménez-Munoz et al. 2021). Often found inhibitors in PBP are Trypsin and chymotrypsin inhibitors, serine protease

inhibitors, aspartic and cysteine proteases, and α-amylase inhibitors as listed in review from Kårlund et al. (2021).

Tannins, for example in beans, peas, barley, millet, are another type of antinutrient and they can bind to proteins and form complexes and precipitates in aqueous environment or exert steric effects due their large size (Hagerman et al. 1992, Duodu et al. 2003, Gilani et al. 2005, Gilani et al. 2012). Seeds, grains, nuts, and beans contain phytic acid which has the ability to chelate with minerals, e.g. calcium, and form protein-cation-protein complexes or bind directly to protein, thus interfering with digestibility in GI tract (Gilani et al. 2005, Brishti et al. 2020). Phytic acid can also interfere with pepsin activity and inhibit digestion (Gilani et al. 2012, Martineau-Côté et al. 2022).

Maillard reaction, advanced glycosylation end-products (AGEs), can reduce protein digestibility in rats (Delgado-Andrade and Fogliano 2018). In humans, the protein utilization was found to be 6 % lower with high MRP diet compared to low MRP diet, which was significant but not seriously compromising the apparent nitrogen absorption (Seiquer et al. 2006). Lysine goes through modifications in the Maillard reaction thus becomes biologically unavailable and induces reactive carbonyls that may react with other AA in high heat conditions (Gilani et al. 2012).

Processing of proteins, e.g., alkaline treatment or severe heat and roasting, may cause racemization where D-amino acids are generated (Duodu et al. 2003, Gilani et al. 2012). Hydrolyzation of proteins occurs at L-amino acid peptide bonds. Therefore, appearance of D-amino acids slows the rate of hydrolysis and allows creation of D-D, D-L, and L-D peptide bonds which are partly or fully inaccessible for digestive enzymes. D-amino acids are absorbed more slowly and in addition, D isomers are poorly utilized by humans (Duodu et al. 2003, Gilani et al. 2012).

### 3.2.4 Plant- vs animal-based proteins

Proteins are stored in plant sources, e.g., as globulins or albumins and the structure of these is different than in animal-based sources as for example the β-sheet content is higher in plant-based sources than in animal-based sources, which make them resistant to digestive enzymes (Carbonaro et al. 2015). Furthermore, structure of the seed and cell wall can prevent proteins from being degraded by acidic environment or enzymes in the GI tract and proteins can also react with non-protein components decreasing digestibility (Duodu et al. 2003).

Also PBPs three-dimensional structure differs from animal-based proteins (Carbonaro et al. 2015). **Figure 3** presents the 3D structures of plant and animal-based proteins. In PBPs there is a high amount of β-sheet conformation whereas animal-based proteins have relatively high amount of α-helix. β-sheet conformation is considered resistant to proteolysis (Carbonaro et al. 2015). For example, β-lactoglobulin that is found in bovine milk and has relatively high amount of β-sheet content which might be related to resistance to GI denaturation (Carbonaro et al. 2015).

Hydrophobic character of β-sheet results in the reduced digestibility of proteins having this conformation, and the amount of β-sheets in content has high linear correlation with digestibility of proteins (Carbonaro et al. 2012).

Taken together the factors that limit the digestibility, PBPs have generally lower digestibility (63–100 %) (**Table 6**) compared to animal-based proteins (90–95 %) (Sá et al. 2020). Furthermore, pepsin is more able to hydrolyze animal-based proteins (Moran 2016).

PBP do not usually fill the IAA requirements recommended by WHO/FAO due to their AA composition (Gorissen et al. 2018). Compared to ABPs, IAA content in PBPs is 11 % lower (37 % vs 26 %, respectively). Typically one of either lysine, threonine, or methionine is the limiting AA in PBP and mixing various PBPs may provide comparable protein content to those in animal-based sources (Elango et al. 2012, Gorissen et al. 2018).

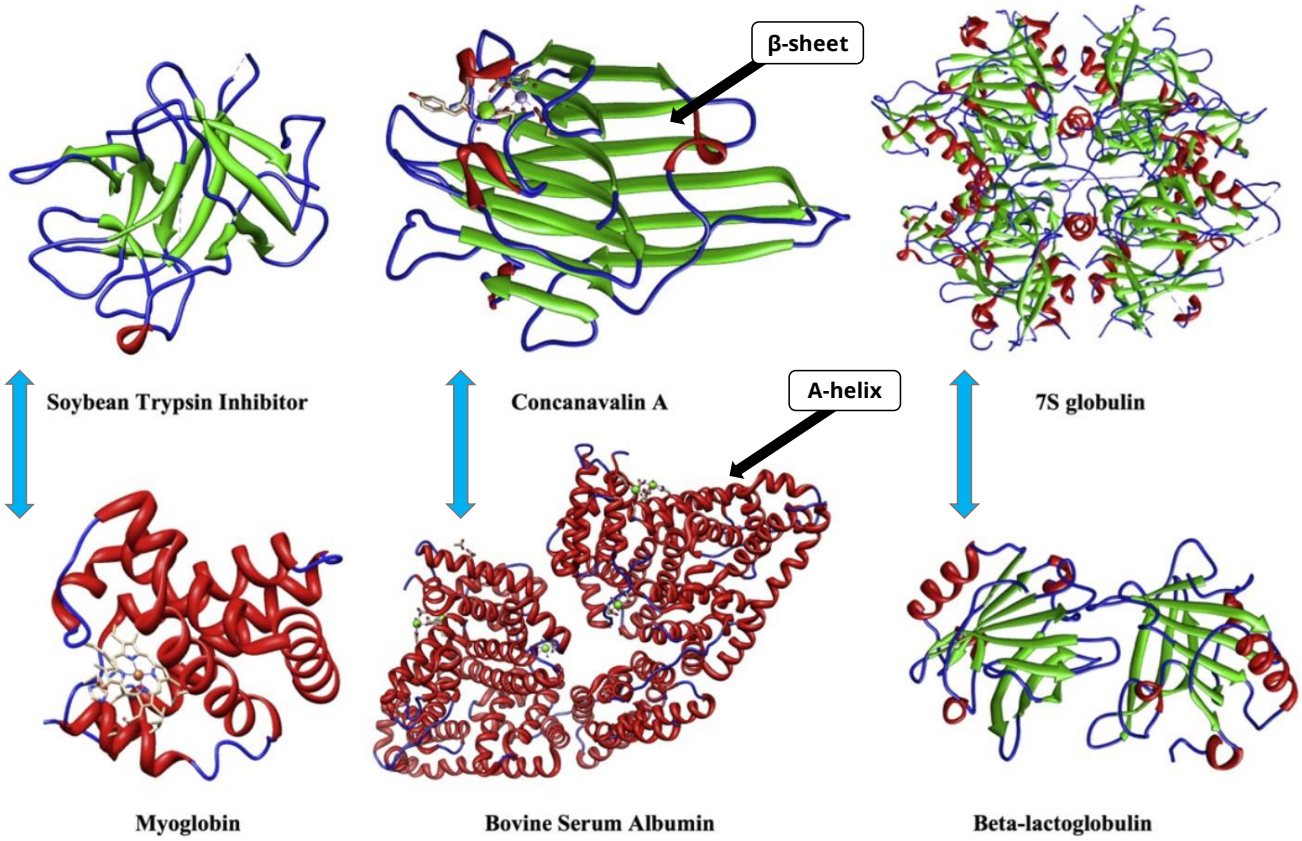


**Figure 3.** Comparison between plant and animal protein structure. The top row is plant based and the bottom row is animal based. Modified from (Carbonaro et al. 2015).

# 4 Methods to study digestibility

## 4.1 In vitro methods

Animal and human studies are often time consuming and expensive, and may present ethical problems (Hur et al. 2011, Sousa et al. 2020, Song et al. 2022). In vitro methods are commonly used to measure digestibility of foods and how proteins withstand digestive processes, and there are several types of in vitro methods used for foodstuff (Coda et al. 2017, Brodkorb et al. 2019). Methods are used to simulate GI tract conditions and can be divided into static and dynamic methods. Static is considered a simple method and the dynamic method is relatively complex and expensive, and therefore not used in research that often (Brodkorb et al. 2019). In vitro models are seen as potential predictors of true digestibility and are useful in estimating in vivo digestion (Bohn et al. 2018).

Although, in vitro measures do not fully resemble digestive physiology, as for example neurohormonal control and intestinal absorption are lacking, and gastric mixing is difficult to mimic, in controlled circumstances it enables accurate determination of monitored parameters and inter-trial variability is lower than in in vivo trials (Reynaud et al. 2020).

### 4.1.1 INFOGEST and related protocols

INFOGEST 2.0 protocol was developed to create international consensus on a set of digestion parameters used in static and semi-dynamic in vitro methods (Brodkorb et al. 2019). Standardization enables better comparability of study results (Bohn et al. 2018). Gastric and intestinal phase is shown to reflect in vivo protein digestion (Bohn et al. 2018). A new standardized semi-dynamic in vitro digestion method consensus statement was published in 2020 (Mulet-Cabero et al. 2020).

The INFOGEST method consists of three steps: preparation, digestion, and sample treatment (Brodkorb et al. 2019). In the first step, preparation, the concentrations of bile salts and digestive

enzymes should be determined experimentally, and this is highlighted as a particularly crucial step. Digestion has three phases including oral, gastric, and intestinal phase. Food is exposed to digestive enzymes mimicking human digestion (Brodkorb et al. 2019). In semi-dynamic protocol, the enzymes are added gradually whereas in static protocol the aim is the correct final ratio, and also the pH is decreased gradually (Mulet-Cabero et al. 2020). The last step is sampling, which should be considered carefully before digestion depending on endpoints, purposes, and type of analysis (Brodkorb et al. 2019).

## 4.2 In vivo methods

Typically, when conducting in vivo animal studies, rats or pigs are used. Pigs have been shown to have close to similar digestion as humans and are considered useful in determination of dietary protein digestibility (Rowan et al. 1994, de Vrese et al. 2000, Brodkorb et al. 2019). Rats and humans have similarities in food digestion; thus, rats are considered as a reasonable approximation of the true digestibility of crude protein digestibility for humans (Food and Agriculture Organization of the United Nations 2013). However, the FAO (Food and Agricultural Organization) report suggests that validation is needed to agree on preferred species for animal tests (Leser 2013).

In animal studies, two common methods used are fecal sample collection, where nitrogen amount is measured from the sample, and T-cannula in distal ileum with $^{15}N$-labeled proteins (de Vrese et al. 2000, Brishti et al. 2020, Reynaud et al. 2021).

Human studies can be carried out by using naso-gastric or naso-intestinal probes to collect samples of digestive effluents (Boutrou et al. 2013). Methods often contain either $^{15}N$-labeled or labeled $^{2}H_2O$ proteins (either ABB or PBP) and they enable differentiation of the dietary and endogenous fractions and determination of real ileal digestibility (Mahé et al. 1992, Kashyap et al. 2019, Devi et al. 2020, Guillin et al. 2021).

Relatively new less invasive and analytically simple technique to determine AA require for humans is developed from indicator amino acid oxidation (IAAO) method and is called metabolic availability (MA) (Moehn et al. 2005, Bandyopadhyay et al. 2022). For PBPs, MA can be determined for individual AAs (Elango et al. 2012). Therefore, it is time consuming and requires compliance from subjects (Bandyopadhyay et al. 2022).

# 5 Plant protein digestibility in vitro and in vivo studies

## 5.1 In vitro studies

The digestibility of PBPs has been researched in several studies. **Table 3** compiles the digestibility values reported in in vitro studies. Digestibility for PBPs varies based on study methods and plant/cereal cultivars are in the range of 64–98 %. Pea is commonly used in studies and digestibility values are in between 70–98 %.

Santos-Hernández et al. (2020) reported digestibility of 98 % for grass and garden pea. Digestibility of soybean did not reach as high as for pea, being 88 % in the previously mentioned study. Lowest values of pea digestibility were reported by Jiménez-Munoz et al. (2022) with 79 % for protein isolate and 70 % for heated (15 min in 90 °C) protein isolate.

Chen et al. (2019) studied soybean meal digestibility, which was 92 %. In the same study digestibility of rapeseed meal and wheat gluten was 79 % and 98 %, respectively. Coda et al. (2017) reported 64 % digestibility for wheat flour.

Nosworthy et al. (2017a) studied three different processing methods, extrusion, cooking, and baking, for yellow and green pea. Digestibility ranged from 82 % to 87 % for yellow pea and 82 % to 90 % for green pea, extrusion giving the highest value and baking (29–35 min in 166–198 °C) the lowest. Digestibility of raw legumes, lentil, chickpea, yellow and green pea, and soybean, increased by 5 %, 8 %, 2 %, 4 %, and 12 %, respectively, after heating 30 min in 98 °C (Han et al. 2007).

Quinoa, barley and mungbean protein isolates were studied by Song et al. (2022) and digestibility of 86 %, 75 % and 64 % was reported, respectively. Digestibility of oat was 87 % according to results by Yuanqing et al. (2020).

**Table 3.** In vitro protein digestibility in studies selected to this review by convenience and full text availability.

| Reference | Protocol | Processing | Source | Protein % | Digestibility (%) | Reference value |
|---|---|---|---|---|---|---|
| Santos-Hernández et al. 2020 (Spain) | INFOGEST | Protein isolate | Garden pea | 77 | 97.8 | Free amino acid content in the soluble phase |
| | | | Grass pea | 82 | 98.2 | |
| | | | Soybean | 63 | 87.9 | |
| | | | Lentil | 75 | 96.2 | |
| Chen et al. 2018 (Netherlands) | Soluble N | | Soybean meal | 46.8 ± 1.0[a] – 48.5 ± 0.3[a] | 92 ± 1[a] | % of total N during the sequential incubation with pepsin and pancreatin |
| | | | Rapeseed meal | 34.4 ± 1.2[a] | 79 | |
| | | | Wheat gluten | 78.1 ± 1.8[a] | 98 | |
| Coda et al. 2017 (Italy) | Solubilized protein | Baking | Wheat flour | 14 ± 2 | 64.1 ± 2.1b | % of the total solubilized protein |
| Song et al. 2022 (China) | Soluble N | Protein isolate | Quinoa | 60.28 | 85.79 | total nitrogen in the soluble fraction and free nitrogen in the soluble fraction |
| | | | Barley | 82.88 | 74.98 | |
| | | | Mung bean | 84.61 | 64.14 | |
| Nosworthy et al. 2017a (Canada) | pH drop measurement | Extrusion | Yellow pea | 24.56 | 86.93 ± 0.1[b] | Not reported |
| | | Cooked | | 22.88 | 86.75 ± 0.63[b] | |
| | | Baked | | 23.35 | 82.49 ± 0.54[b] | |
| | | Extrusion | Green pea | 25.08 | 90 ± 0.81[b] | |
| | | Cooked | | 23.91 | 86.56 ± 0.45[b] | |
| | | Baked | | 22.77 | 82.22 ± 0.63[b] | |
| Han et al. 2007 (USA) | Three enzyme method | Raw/Heated | Lentil | | 79.1/83.6 | Soluble proteins and insoluble tailings |
| | | | Chickpea | | 74.3/82.5 | |
| | | | Yellow pea | | 82/84.0 | |
| | | | Green pea | | 82.6/84.1 | |
| | | | Soybean | | 71.8/83.8 | |
| Yuanqing et al. 2020 (China) | - | Extracted | Oat | 68.63 | 86.58 | Protein content in the sample |

N=nitrogen. Superscript a = standard error of the mean, superscript b = standard deviation.

In **Table 4** are three studies summarized that measured degree of hydrolysis (DH) with static or semi-dynamic in vitro method. By using static method Reynaud et al. (2020) studied DH for seitan, tofu and soy. Seitan and tofu were either fresh or freeze dried. DH for fresh seitan was 11,8 % and for freeze dried 26,3 %. DH for fresh tofu was 33,1 % and freeze dried 33,9 %.

**Table 4.** In vitro degree of hydrolysis (DH).

| | | | | **DH (%)** | |
|---|---|---|---|---|---|
| **Reference** | **Protocol** | **Source** | **Processing** | **GA + Int** | **Reference value** |
| Reynaud et al. 2020 (France) | INFOGEST | Seitan, fresh | Ground < 2 mm | 11.8 ± 2.6[a] | Total N in food – concentration of α-amino acids before addition of pepsin |
| | | Seitan, freeze dried | | 26.3 ± 0.9[a] | |
| | | Tofu, fresh | pH precipitation | 33.1 ± 4.0[a] | |
| | | Tofu, freeze dried | | 33.9 ± 6.7[a] | |
| | | Soy milk-related protein isolate | | 17.9 ± 1.8[a] | |
| | | Soy milk | | 50.3 ± 7.6[a] | |
| Rivera del Rio et al. 2022 (Netherlands) | Semi-dynamic | Yellow pea | Protein isolate | 74 | Total peptide bonds |
| | | Yellow pea | Protein isolate (heated, 90°C, 15-32 min) | 71 | |
| Jiménez-Munoz et al. 2022 (Denmark) | INFOGEST | Pea | Protein isolate | 78.60 ± 0.47 | The % of soluble protein relative to the total protein content in the initial protein dispersion. |
| | | | Protein isolate (heated, 90°C, 15 min) | 70.33 ± 3.5 | |

GA = gastric digestion, GA + Int = gastric + intestinal digestion, superscript a = standard deviation.

Unheated and heated yellow pea protein isolates were studied by Rivera del Rio et al. (2022) using semi-dynamic in vitro method and DH of unheated was 74 % and heated 71 %, though after gastric phase there was no significant difference in DH between unheated and heated.

DH for soy milk was 51 % whereas soy milk-related protein isolate had 18 % DH (Reynaud et al. 2020). Authors also studied DH of pea emulsion (not included in the table) and after GI digestion the DH was 73,6 ± 12,1 %.

## 5.2 In vivo studies

### 5.2.1 Animal studies

Digestibility of PBPs is being studied commonly in animals, mostly in rats and pigs. PBP digestibility is in range of 64–99 % according to the studies listed in **Table 5**.

Nosworthy et al. (2018) studied digestibility of black, kidney and faba bean in rats using three different processing methods, extrusion, cooking, and baking. Extruded beans had true protein digestibility (TPD) of 82 %, 83 % and 88 % and cooked 82 %, 81 % and 88 %, respectively. The TPD of baked beans was 64 % for black, 69 % for kidney and 89 % for faba beans. (Nosworthy et al. 2017b) reported TPD of 70 % for soaked and cooked black bean. Brishti et al. (2020) found that the TPD of mung bean was 99 % using similar methods, except that the mung beans were freeze-dried prior to extrusion.

Digestibility of soy as isolate, flour, milk, or tofu was reported in four different studies. Soy protein isolate digestibility in rats was 92–97 % (Han et al. 2014; Rutherfurd et al. 2015) and in pigs 94 % (Mathai et al. 2017). Soy flour SID was 92 % (Mathai et al. 2017). Reynaud et al. (2021) reported TID values of 95 % and 92% for soy in the form of tofu and milk, respectively.

Pea digestibility was reported to be between 85 and 98 %. Reported digestibility for differently processed peas were protein concentrate 95–98 %, cooked 85–92 %, baked 87–88 % and extruded protein 91 % (Rutherfurd et al. 2015; Mathai et al. 2017; Nosworthy et al. 2017a; Nosworthy et al. 2017b). Pea isolate emulsion TID was 94 % (Reynaud et al. 2021).

Digestibility for rice in rats was studied in three different forms: bran protein, protein concentrate and cooked. The digestibility values were 95, 82 and 77 %, respectively (Han et al. 2014; Rutherfurd et al. 2015).

**Table 5.** Digestibility values in animal studies.

| Reference | Source | Processing | Subjects | Methods | Digestibility (%) | Reference value |
|---|---|---|---|---|---|---|
| Nosworthy et al. 2018 (Canada) | Faba bean | Baking, cooking, extrusion | Rats | Fecal N loss | 87.60-88.63[d] | Nitrogen intake |
| | Kidney bean | | | | 69.12-83.21[d] | |
| | Black bean | | | | 63.55-82.01[d] | |
| Brishti et al. 2020 (Malaysia) | Mung bean | Extrusion | Rats | Fecal N loss | 99.26[e] | Nitrogen intake |
| Han et al. 2014 (Republic of Korea) | Soy | Isolated protein | Rats | Fecal N loss | 91.7[e] | Nitrogen intake |
| | Rice | Bran protein | | | 94.8[e] | |
| Nosworthy et al. 2017b (Canada) | Black bean | Soaked & cooked | Rats | Fecal N loss | 69.99[e] | Nitrogen intake |
| | Green lentil | Cooked | | | 87.89[e] | |
| | Split red lentil | Cooked | | | 90.6[e] | |
| | Yellow pea | Cooked | | | 87.94[e] | |
| | Green pea | Cooked | | | 85.15[e] | |
| Rutherfurd et al. 2015 (New Zealand) | Pea | Protein concentrate | Rats | Fecal N loss (days 11-14) & terminal ileal sample at the end of the study (day 15) from dead rats | 98[d] | mg of the ith dietary indispensable amino acid in 1 g of the reference protein [i (1-n) = dietary indispensable or conditionally indispensable AA]* |
| | Pea | Cooked | | | 92[d] | |
| | Rice | Protein concentrate | | | 82[d] | |
| | Rice | Cooked | | | 77[d] | |
| | Rolled oats | Cooked | | | 87[d] | |
| | Wheat bran | - | | | 74[d] | |
| | Soy | Protein isolate | | | 97[d] | |
| | Kidney bean | Cooked | | | 77[d] | |
| Reynaud et al. 2021 (France) | Pea | Protein isolate emulsion | Miniature pigs | Catheters and T-cannula | 94.2 ± 1.3[ad] | AA intake |
| | Soybean | Milk, UHT | | | 92.3 ± 3.0[ad] | |

(continues)

Table 5, continues

| Reference | Source | Processing | Subjects | Methods | Digestibility (%) | Reference value |
|---|---|---|---|---|---|---|
| Nosworthy et al. 2017a (Canada) | Yellow pea | Extrusion | Rats | Fecal N loss | 91.35 ± 2.2[be] | Nitrogen intake |
| | | Cooked | | | 89.00 ± 1.8[be] | |
| | | Baked | | | 86.77 ± 2.6[be] | |
| | Green pea | Extrusion | | | 90.73 ± 2.8[be] | |
| | | Cooked | | | 87.58 ± 1.6[be] | |
| | | Baked | | | 88.51 ± 2.2[be] | |
| Berrazaga et al. 2020 (France) | FB (62 %)-enriched pasta (38 %) | Cooked | Old rats | Fecal N loss | 92.85 ± 0.97[ae] | Nitrogen intake |
| | L (65 %)-enriched pasta (35 %) | | | | 85.44 ± 1.90[ae] | |
| | SP (79 %)-enriched pasta (21 %) | | | | 93.02 ± 0.84[ae] | |
| Reynaud et al. 2021 (France) | Seitan, freeze dried | Ground 3,5 ± 0,1 mm | Pigs | Catheters and T-cannula | 97.5 ± 1.5[ad] | AA intake |
| | Tofu, freeze dried | | | | 95.0 ± 2.3[ad] | |
| de Vrese et al. 2000 (Germany) | Wheat protein | Native | Miniature pigs | $^{15}$N-labeled protein & T-cannula | 93.4 ± 0.2[ae] | AA intake |
| | | Heated (24 h, 65°C, pH 11.5) | | | 75.6 ± 0.7[ae] | |
| Mathai et al. 2017 (USA) | Pea | Protein concentrate | Pigs | T-cannula in distal ileum | 95 ± 1.8[ac] | AA intake** |
| | Soy | Protein isolate | Pigs | | 94 ± 0.6[ac] | |
| | Soy | Flour | Pigs | | 92 ± 3.1[ac] | |
| | Wheat | Flour | Pigs | | 91 ± 4.5[ac] | |

N=nitrogen. Superscripts: a = standard error of the mean, b = standard deviation, c = standard ileal digestibility, d = true ileal digestibility, e = true protein digestibility. FB = faba bean, L = lentil, SP = split pea. *AA requirement pattern for the 0.5–3 -years old child. **Method from Stein et al. (2007).

Wheat digestibility in pigs was reported to range from 76 % (de Vrese et al. 2000) to 98 % (Reynaud et al. 2021) with the highest value observed in freeze-dried seitan and the lowest in heated wheat protein. In rats, wheat bran TID was 74 %. Additionally, TID of rolled oats was found to be 87 % by Rutherfurd et al. (2015).

Nosworthy et al. (2017b) studied TPD for cooked whole green lentil and split red lentil in rats and results were 88 and 91 %, respectively. Berrazaga et al. (2020) examined TPD of mixed PBP meals. Faba bean, lentil and split pea were made as pasta with wheat (38, 35 and 21 % of wheat, respectively) and then cooked. The digestibility of the meals were 93 % for faba bean, 85 % lentil and 93 % for split pea enriched pasta (Berrazaga et al. 2020).

### 5.2.2 Human studies

Digestibility of common PBPs from human studies are presented in **Table 6**. Reported values were between 63 % and 100 %. The digestibility of soy was reported in four studies and it ranges from 91 % to 100 % depending on study method (Wang et al. 1983, Mariotti et al. 1999, Gaudichon et al. 2002). Mariotti et al. (1999) and Gaudichon et al. (2002) had similar methods and processing except measured factor, and the values of soy digestibility were 91 % and 94 %, respectively.

The digestibility of yellow pea was reported to be 72–94 %. Cooked yellow pea was used in two studies but the methods used to estimate digestibility were different. Gausserès et al. (1996) reported 89 % GI absorption for cooked yellow pea and Kashyap et al. (2019) reported 72 % TID of pressure cooked yellow pea. Digestibility of extruded yellow pea was 86 % (Devi et al. 2020). TID of pressure cooked chickpea by Kashyap et al. (2019) was 75 % whereas Devi et al. (2020) reported 88 % digestibility of extruded chickpea. Kashyap et al. studied also mung pea in two different forms, pressure cooked and dehulled pressure cooked, and the TIDs were 63 % and 71 %, respectively.

**Table 6.** Digestibility values in human studies.

| Reference | Source | Method | Processing | n | Mean age | Measurement method | Digestibility | Reference value |
|---|---|---|---|---|---|---|---|---|
| Gaudichon et al. 2002 (Germany) | Soy | $^{15}$N-labeled | Protein isolate | 6 | 28 ± 8[a] | Ileal tube | 93.8 ± 3.0[ae] | Nitrogen and AA intake |
| Wang et al. 1983 (Japan) | Soy | Feces from last 8 days | Protein isolate | 5 | 24 ± 3[a] | Fecal N loss | 99.8-100[d] | Nitrogen intake |
| | Soybean | - | Cooked | | - | - | 94[d]* | |
| Mariotti et al. 1999 (France) | Soy | $^{15}$N-labeled | Protein isolate | 10 | 28 ± 5[a] | Ileal tube | 90.9 ± 2.2[ah] | Not reported |
| Gausserès et al. 1996 (France) | Yellow Pea | $^{15}$N-labeled | Cooked | 7 | 28 ± 7[a] | Ileal tube | 89.4 ± 1.1[af] | $^{15}$N-labeled pea intake |
| Devi et al. 2020 (India) | Chickpea | Labeled $^{2}H_2O$ | Extrusion | 14 | 8.4 ± 0.9[a] | Blood sample | 87.5 ± 9.4[ad] | Calculated in ratio to a standard protein, [U-$^{13}$C] spirulina. |
| | Yellow pea | | | 14 | 8.7 ± 0.7[a] | | 86.0 ± 11.2[ad] | |
| Kashyap et al. 2019 (India) | Chickpea | Labeled $^{2}H_2O$ | Pressure cooked | 6 | 23.8 ± 2.2[a] | Blood sample | 74.6 ± 0.8[ab] | Calculated in ratio to a standard protein, [U-$^{13}$C] spirulina. |
| | Yellow pea | | | 6 | 22.8 ± 3.5[a] | | 71.6 ± 1.3[ab] | |
| | Mung bean (MB) | | | 5 | 24.6 ± 3.9[a] | | 63.2 ± 1.5[ab] | |
| | MB dehulled | | | 6 | 23.5 ± 3.8[a] | | 70.9 ± 2.1[ab] | |

N=nitrogen. Superscripts: a=standard deviation, b=true ilea IAA digestibility, c=true protein digestibility, d=digestibility, e=amino acid digestibility, f=gastrointestinal absorption, g=true oro-ileal digestibility, h=real ileal digestibility. *No access to study itself and value is obtained from Wang et al. (1983).

# 6 Discussion

In this review digestibility of common PBP sources was examined based on the current literature. Overall, it has been shown that PBPs have good digestibility which can be further improved through processing. To break down molecular structure of PBPs is challenging for the digestive enzymes compared to animal sources. PBP digestibility was reported to be 63–100 %.

Variation in results is partly due to different research methods, e.g., fecal sample or ileal tube, and measured factors, e.g., SID vs TPD. Processing of the studied protein also affects results, as it can change the protein structure and thus make proteins more accessible to digestive enzymes or deactivate antinutritional factors. For example, cooking of wheat delayed digestion (unlike with pea) and it could be due cross-linking with the matrix in gastric phase (Assad-Bustillos et al. 2020).

In studies using in vitro methods, PBP digestibility was between 64 % and 98 %. Santos-Hernández et al. (2020) reported digestibility of garden and grass pea, and lentil to be 98 % and 96 %, respectively. Authors noted that high processing of foods removed antinutritional components from protein isolates, thus increasing the digestibility comparable to animal proteins. Interestingly, digestibility of soybean protein isolate in the same study was 88 % and that might have been due aggregation tendency of disulfide bonds in glycinin, which is main storage protein in soy (Santos-Hernández et al. 2020). Similar results were also observed by Reynaud et al. (2020) using DH method where DH for tofu was 33 % whereas soy milk had 51 % DH. Authors noted that β-conglycinin, which is absent in soymilk, undergoes structural changes in gastric conditions and decreases the solubility and further influence intestinal digestion of soy.

Comparison of the results between studies is rather difficult due to variation in methods and different processing. For example, many studies have examined the digestibility of peas and reported digestibility 70–98 % depending on used study and processing methods. Thus, processing seems to influence digestibility values. The lowest in vitro digestibility value was for heated pea protein isolate. Heating can decrease the digestibility or DH due coagulation of

storage proteins (Jiménez-Munoz et al. 2021) as was observed by Nosworthy et al. (2017a) and Jiménez-Munoz et al. (2022) with heated or baked pea having 4–8 % lower digestibility or DH than unheated. Though, heating of raw legumes increased their digestibility 2–12 % (Han et al. 2007). This could be due to raw legume in comparison of protein isolate and also different processing method or heating prior to measurement. Lower digestibility was also observed in rats for cooked pea and rice compared to concentrates, 6 % and 5 %, respectively (Rutherfurd et al. 2015). One explanation for decreased digestibility of heated food could be the gelatinization due to heating that might cause polar and non-polar interactions with starch proteins, thus decreasing the digestibility (Rivera del Rio et al. 2022). Nevertheless, heating may cause significant differences in breakdown kinetics of the pea protein during digestion, thus impacting bioavailability (Jiménez-Munoz et al. 2022).

In human studies, the difference in digestibility of pressure-cooked yellow pea was 14 % lower (Kashyap et al. 2019) compared to extruded yellow pea (Devi et al. 2020), based on similar study methods. Difference could be explained by the processing and administration of the protein or the age of the test subjects (24 vs 9 years, respectively), or a combination of both. Kashyap et al. (2019) used a test meal of which two thirds of total protein amount was the test protein thus there might have been food matrix effects. Other components in food have been shown to delay or impair digestibility of proteins (Torcello-Gómez et al. 2020), which decreases the digestibility. In contrast, Devi et al. (2020) used tablet which contained only the test protein. In these two studies, the digestibility of chickpea was similar to yellow pea, being 13 % lower (Kashyap et al. 2019, Devi et al. 2020). In addition, Wang et al. (1983) showed similar difference with soy protein isolate in comparison to cooked soybean. It has to be noted though, that fecal nitrogen loss method, which was used by Wang et al. (1983), does not take into account AA that are not absorbed in the small intestine but used in the colon (Schaafsma 2012), thus potentially overestimating the nitrogen absorption. De Vrese et al. (2000) reported noticeable difference of wheat protein TPD in pigs between processing methods where heated wheat protein had 17 % lower digestibility than native protein.

Even though, digestibility of PBPs does not reach the extent of animal protein digestibility, they can be considered good protein sources as reported digestibility values reached over 95 %, especially if selected processing methods can enhance the digestibility, e.g., extrusion or isolated proteins. The lowest digestibility reported in vivo studies was 63 % for pressure cooked mung bean whereas for alkaline extracted and extruded mung bean the digestibility was 99 % which demonstrated that processing of mung bean can enhance the digestibility significantly by inactivating the antinutritional factors (Brishti et al. 2020). Santos-Hernández et al. (2020) reported comparable results for peas (98 %) and lentil (96 %) regarding the removal of antinutritional factors, that PBPs can reach almost equal digestibility as animal-based sources.

Digestibility of mixed PBPs was studied in older rats by Berrazaga et al. (2020) where they compared digestibility of legume-based pasta to casein and skim milk powder (SMP). Rats fed with animal-based proteins had significantly higher true digestibility compared to legume-enriched pasta fed rats. Net protein utilization for rats fed legume pasta was comparable to rats fed with casein. Skeletal muscle mass was similar for pea-enriched pasta and SMP whereas casein and faba bean pasta and lentil pasta had similar skeletal muscle but lower compared to P-pasta or SMP. Lentil pasta showed 21 % higher trypsin inhibitory than pea pasta and 11–14 % higher amount disulfide-linked proteins than other two which can limit GI enzymes access and activity thus decreasing digestibility (Berrazaga et al. 2020).

# 7 Conclusion

In this work, the literature of digestibility of PBPs in in vitro and in animals and humans was reviewed. Quantitative comparison of digestibility was not possible due to great variation of methods and processing used to measure digestibility. The digestibility of plant proteins is affected by many factors, such as plant cell wall structures or antinutrients. However, processing can increase the digestibility and bioaccessibility of the PBP by breaking the cell wall structure and inactivating some of the antinutrients.

In conclusion, digestibility of PBP was reported to be in the range of 63–100 %, demonstrating large variability in digestive capability of PBP in the gastrointestinal tract. Thus, plants can be considered a suitable source of protein for human nutrition, especially when consumed in mixtures.

## References

Acquah C, Zhang Y, Dubé MA, Udenigwe CC. Formation and characterization of protein-based films from yellow pea (Pisum sativum) protein isolate and concentrate for edible applications. Curr Res Food Sci 2020;2:61–69. doi:10.1016/j.crfs.2019.11.008

Amagliani L, O'Regan J, Kelly AL, O'Mahony JA. Composition and protein profile analysis of rice protein ingredients. J Food Compos Anal 2017;59:18–26. doi:10.1016/j.jfca.2016.12.026

Assad-Bustillos M, Palier J, Rabesona H, Choiset Y, Della Valle G, Feron G. Role of the bolus degree of structure on the protein digestibility during in vitro digestion of a pea protein-fortified sponge cake chewed by elderly. J Texture Stud 2020;51:134–143. doi:10.1111/jtxs.12486

Bandyopadhyay S, Kashyap S, Calvez J, et al. Evaluation of Protein Quality in Humans and Insights on Stable Isotope Approaches to Measure Digestibility – A Review. Adv Nutr 2022;13:1131–1143. doi:10.1093/advances/nmab134

Berrazaga I, Salles J, Laleg K, et al. Anabolic Properties of Mixed Wheat-Legume Pasta Products in Old Rats: Impact on Whole-Body Protein Retention and Skeletal Muscle Protein Synthesis. Nutrients 2020;12:1596. doi:10.3390/nu12061596

Bohn T, Carriere F, Day L, et al. Correlation between in vitro and in vivo data on food digestion. What can we predict with static in vitro digestion models? Crit Rev Food Sci Nutr 2018;58:2239–2261. doi:10.1080/10408398.2017.1315362

Boutrou R, Gaudichon C, Dupont D, et al. Sequential release of milk protein–derived bioactive peptides in the jejunum in healthy humans. Am J Clin Nutr 2013;97:1314–1323. doi:10.3945/ajcn.112.055202

Brishti FH, Chay SY, Muhammad K, Rashedi Ismail-Fitry M, Zarei M, Saari N. Texturized mung bean protein as a sustainable food source: techno-functionality, anti-nutrient properties, *in vivo* protein quality and toxicity. Food Funct 2020;11:8918–8930. doi:10.1039/D0FO01463J

Brodkorb A, Egger L, Alminger M, et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc 2019;14:991–1014. doi:10.1038/s41596-018-0119-1

Carbonaro M, Maselli P, Nucara A. Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: a Fourier transform infrared (FT-IR) spectroscopic study. Amino Acids 2012;43:911–921. doi:10.1007/s00726-011-1151-4

Carbonaro M, Maselli P, Nucara A. Structural aspects of legume proteins and nutraceutical properties. Food Res Int 2015;76:19–30. doi:10.1016/j.foodres.2014.11.007

Chen H, Wierenga PA, Hendriks WH, Jansman AJM. In vitro protein digestion kinetics of protein sources for pigs. Animal 2019;13:1154–1164. doi:10.1017/S1751731118002811

Coda R, Varis J, Verni M, Rizzello CG, Katina K. Improvement of the protein quality of wheat bread through faba bean sourdough addition. LWT - Food Sci Technol 2017;82:296–302. doi:10.1016/j.lwt.2017.04.062

Delgado-Andrade C, Fogliano V. Dietary Advanced Glycosylation End-Products (dAGEs) and Melanoidins Formed through the Maillard Reaction: Physiological Consequences of their Intake. Annu Rev Food Sci Technol 2018;9:271–291. doi:10.1146/annurev-food-030117-012441

Devi S, Varkey A, Dharmar M, et al. Amino Acid Digestibility of Extruded Chickpea and Yellow Pea Protein is High and Comparable in Moderately Stunted South Indian Children with Use of a Dual Stable Isotope Tracer Method. J Nutr 2020;150:1178–1185. doi:10.1093/jn/nxaa004

Dizlek H, Awika JM. Determination of basic criteria that influence the functionality of gluten protein fractions and gluten complex on roll bread characteristics. Food Chem 2023;404:134648. doi:10.1016/j.foodchem.2022.134648

Duodu KG, Taylor JRN, Belton PS, Hamaker BR. Factors affecting sorghum protein digestibility. J Cereal Sci 2003;38:117–131. doi:10.1016/S0733-5210(03)00016-X

Elango R, Levesque C, Ball RO, Pencharz PB. Available versus digestible amino acids – new stable isotope methods. Br J Nutr 2012;108:S306–S314. doi:10.1017/S0007114512002498

Emkani M, Oliete B, Saurel R. Pea Protein Extraction Assisted by Lactic Fermentation: Impact on Protein Profile and Thermal Properties. Foods 2021;10:549. doi:10.3390/foods10030549

Espinosa-Marrón A, Adams K, Sinno L, et al. Environmental Impact of Animal-Based Food Production and the Feasibility of a Shift Toward Sustainable Plant-Based Diets in the United States. Front Sustain 2022;3:841106. doi:10.3389/frsus.2022.841106

Food and Agriculture Organization of the United Nations. (toim.). Dietary protein quality evaluation in human nutrition: report of an FAO expert consultation, 31 March-2 April 2011, Auckland, New Zealand. Rome: Food and Agriculture Organization of the United Nations 2013.

Fujiwara T, Nambara E, Yamagishi K, Goto DB, Naito S. Storage Proteins. Arab Book 2002;1:e0020. doi:10.1199/tab.0020

Gaudichon C, Bos C, Morens C, et al. Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. Gastroenterology 2002;123:50–59. doi:10.1053/gast.2002.34233

Gausserès N, Mahé S, Benamouzig R, et al. The gastro-ileal digestion of $^{15}$ N-labelled pea nitrogen in adult humans. Br J Nutr 1996;76:75–85. doi:10.1079/BJN19960010

Ghosh S, Suri D, Uauy R. Assessment of protein adequacy in developing countries: quality matters. Br J Nutr 2012;108:S77–S87. doi:10.1017/S0007114512002577

Gilani GS, Cockell KA, Sepehr E. Effects of Antinutritional Factors on Protein Digestibility and Amino Acid Availability in Foods. J AOAC Int 2005;88:967–987. doi:10.1093/jaoac/88.3.967

Gorissen SHM, Crombag JJR, Senden JMG, et al. Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids 2018;50:1685–1695. doi:10.1007/s00726-018-2640-5

Guillin FM, Gaudichon C, Guérin-Deremaux L, et al. Multi-criteria assessment of pea protein quality in rats: a comparison between casein, gluten and pea protein alone or supplemented with methionine. Br J Nutr 2021;125:389–397. doi:10.1017/S0007114520002883

Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArthur C. Tannin Chemistry in Relation to Digestion. J Range Manag 1992;45:57. doi:10.2307/4002526

Han lH, Swanson BG, Baik B-K. Protein Digestibility of Selected Legumes Treated with Ultrasound and High Hydrostatic Pressure During Soaking. Cereal Chem J 2007;84:518–521. doi:10.1094/CCHEM-84-5-0518

Han S-W, Chee K-M, Cho S-J. Nutritional quality of rice bran protein in comparison to animal and vegetable protein. Food Chem 2015;172:766–769. doi:10.1016/j.foodchem.2014.09.127

Hur SJ, Lim BO, Decker EA, McClements DJ. In vitro human digestion models for food applications. Food Chem 2011;125:1–12. doi:10.1016/j.foodchem.2010.08.036

Jiménez-Munoz L, Brodkorb A, Gómez-Mascaraque LG, Corredig M. Effect of heat treatment on the digestion behavior of pea and rice protein dispersions and their blends, studied using the semi-dynamic INFOGEST digestion method. Food Funct 2021;12:8747–8759. doi:10.1039/D1FO01223A

Jiménez-Munoz L, Torp Nielsen M, Roman L, Corredig M. Variation of in vitro digestibility of pea protein powder dispersions from commercially available sources. Food Chem 2023;401:134178. doi:10.1016/j.foodchem.2022.134178

Kashyap S, Varkey A, Shivakumar N, et al. True ileal digestibility of legumes determined by dual-isotope tracer method in Indian adults. Am J Clin Nutr 2019;110:873–882. doi:10.1093/ajcn/nqz159

Kimura A, Fukuda T, Zhang M, Motoyama S, Maruyama N, Utsumi S. Comparison of Physicochemical Properties of 7S and 11S Globulins from Pea, Fava Bean, Cowpea, and French Bean with Those of Soybean—French Bean 7S Globulin Exhibits Excellent Properties. J Agric Food Chem 2008;56:10273–10279. doi:10.1021/jf801721b

Kumar M, Tomar M, Potkule J, et al. Advances in the plant protein extraction: Mechanism and recommendations. Food Hydrocoll 2021;115:106595. doi:10.1016/j.foodhyd.2021.106595

Kårlund A, Paukkonen I, Gómez-Gallego C, Kolehmainen M. Intestinal Exposure to Food-Derived Protease Inhibitors: Digestion Physiology- and Gut Health-Related Effects. Healthcare 2021;9:1002. doi:10.3390/healthcare9081002

Leser S. The 2013 FAO report on dietary protein quality evaluation in human nutrition: Recommendations and implications: FAO dietary protein report. Nutr Bull 2013;38:421–428. doi:10.1111/nbu.12063

Lu ZX, He JF, Zhang YC, Bing DJ. Composition, physicochemical properties of pea protein and its application in functional foods. Crit Rev Food Sci Nutr 2020;60:2593–2605. doi:10.1080/10408398.2019.1651248

Mahé S, Huneau J, Marteau P, Thuillier F, Tomé D. Gastroileal nitrogen and electrolyte movements after bovine milk ingestion in humans. Am J Clin Nutr 1992;56:410–416. doi:10.1093/ajcn/56.2.410

Mariotti F, Mahé S, Benamouzig R, et al. Nutritional Value of [15N]-Soy Protein Isolate Assessed from Ileal Digestibility and Postprandial Protein Utilization in Humans. J Nutr 1999;129:1992–1997. doi:10.1093/jn/129.11.1992

Martineau-Côté D, Achouri A, Karboune S, L'Hocine L. Faba Bean: An Untapped Source of Quality Plant Proteins and Bioactives. Nutrients 2022;14:1541. doi:10.3390/nu14081541

Mathai JK, Liu Y, Stein HH. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br J Nutr 2017;117:490–499. doi:10.1017/S0007114517000125

Mendoza EMT, Adachi M, Bernardo AEN, Utsumi S. Mungbean [ *Vigna radiata* (L.) Wilczek] Globulins: Purification and Characterization. J Agric Food Chem 2001;49:1552–1558. doi:10.1021/jf001041h

Moehn S, Bertolo RFP, Pencharz PB, Ball RO. Development of the Indicator Amino Acid Oxidation Technique to Determine the Availability of Amino Acids from Dietary Protein in Pigs ,. J Nutr 2005;135:2866–2870. doi:10.1093/jn/135.12.2866

Mohanta T, Kamran M, Omar M, Anwar W, Choi G. A Database for the Molecular Weight and Isoelectric Points of The Plant Proteomes.

Moran ET. Gastric digestion of protein through pancreozyme action optimizes intestinal forms for absorption, mucin formation and villus integrity. Anim Feed Sci Technol 2016;221:284–303. doi:10.1016/j.anifeedsci.2016.05.015

Mota da Silva AM, Souza Almeida F, Kawazoe Sato AC. Functional characterization of commercial plant proteins and their application on stabilization of emulsions. J Food Eng 2021;292:110277. doi:10.1016/j.jfoodeng.2020.110277

Mulet-Cabero A-I, Egger L, Portmann R, et al. A standardised semi-dynamic *in vitro* digestion method suitable for food – an international consensus. Food Funct 2020;11:1702–1720. doi:10.1039/C9FO01293A

Mäkinen OE, Sozer N, Ercili-Cura D, Poutanen K. Protein From Oat. Teoksessa: Sustainable Protein Sources. Elsevier 2017 , s. 105–119.

Nosworthy M, Medina G, Franczyk A, et al. Effect of Processing on the In Vitro and In Vivo Protein Quality of Beans (Phaseolus vulgaris and Vicia Faba). Nutrients 2018;10:671. doi:10.3390/nu10060671

Nosworthy MG, Franczyk AJ, Medina G, et al. Effect of Processing on the *in Vitro* and *in Vivo* Protein Quality of Yellow and Green Split Peas ( *Pisum sativum* ). J Agric Food Chem 2017a;65:7790–7796. doi:10.1021/acs.jafc.7b03597

Nosworthy MG, Neufeld J, Frohlich P, Young G, Malcolmson L, House JD. Determination of the protein quality of cooked Canadian pulses. Food Sci Nutr 2017b;5:896–903. doi:10.1002/fsn3.473

Pantoja-Uceda D, Palomares O, Bruix M, et al. Solution Structure and Stability against Digestion of rproBnIb, a Recombinant 2S Albumin from Rapeseed: Relationship to Its Allergenic Properties †. Biochemistry 2004;43:16036–16045. doi:10.1021/bi048069x

Pearsall WH, Ewing J. XLIV. THE ISOELECTRIC POINTS OF SOME PLANT PROTEINS. 1924

Perez-Gregorio MR, Días R, Mateus N, de Freitas V. Identification and characterization of proteolytically resistant gluten-derived peptides. Food Funct 2018;9:1726–1735. doi:10.1039/C7FO02027A

Pinckaers PJM, Trommelen J, Snijders T, van Loon LJC. The Anabolic Response to Plant-Based Protein Ingestion. Sports Med 2021;51:59–74. doi:10.1007/s40279-021-01540-8

Rasane P, Jha A, Sabikhi L, Kumar A, Unnikrishnan VS. Nutritional advantages of oats and opportunities for its processing as value added foods - a review. J Food Sci Technol 2015;52:662–675. doi:10.1007/s13197-013-1072-1

Reynaud Y, Buffière C, Cohade B, et al. True ileal amino acid digestibility and digestible indispensable amino acid scores (DIAASs) of plant-based protein foods. Food Chem 2021;338:128020. doi:10.1016/j.foodchem.2020.128020

Reynaud Y, Lopez M, Riaublanc A, Souchon I, Dupont D. Hydrolysis of plant proteins at the molecular and supra-molecular scales during in vitro digestion. Food Res Int 2020;134:109204. doi:10.1016/j.foodres.2020.109204

Rivera del Rio A, Möller AC, Boom RM, Janssen AEM. In vitro gastro-small intestinal digestion of conventional and mildly processed pea protein ingredients. Food Chem 2022;387:132894. doi:10.1016/j.foodchem.2022.132894

Rowan AM, Moughan PJ, Wilson MN, Maher K, Tasman-Jones C. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. Br J Nutr 1994;71:29–42. doi:10.1079/BJN19940108

Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ. Protein Digestibility-Corrected Amino Acid Scores and Digestible Indispensable Amino Acid Scores Differentially Describe Protein Quality in Growing Male Rats. J Nutr 2015;145:372–379. doi:10.3945/jn.114.195438

S. Alghamd S. Chemical Composition of Faba Bean (Vicia faba L.) Genotypes under Various Water Regimes. Pak J Nutr 2009;8:477–482. doi:10.3923/pjn.2009.477.482

Sá AGA, Moreno YMF, Carciofi BAM. Food processing for the improvement of plant proteins digestibility. Crit Rev Food Sci Nutr 2020;60:3367–3386. doi:10.1080/10408398.2019.1688249

Santos-Hernández M, Alfieri F, Gallo V, et al. Compared digestibility of plant protein isolates by using the INFOGEST digestion protocol. Food Res Int 2020;137:109708. doi:10.1016/j.foodres.2020.109708

Sarwar Gilani G, Wu Xiao C, Cockell KA. Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on Protein Quality. Br J Nutr 2012;108:S315–S332. doi:10.1017/S0007114512002371

Schaafsma G. Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. Br J Nutr 2012;108:S333–S336. doi:10.1017/S0007114512002541

Seiquer I, Díaz-Alguacil J, Delgado-Andrade C, et al. Diets rich in Maillard reaction products affect protein digestibility in adolescent males aged 11–14 y1–3. Am J Clin Nutr 2006;83:1082–1088. doi:10.1093/ajcn/83.5.1082

Song B, An L, Han Y, et al. Transcriptome Profile of Near-Isogenic Soybean Lines for β-Conglycinin α-Subunit Deficiency during Seed Maturation. PLOS ONE 2016;11:e0159723. doi:10.1371/journal.pone.0159723

Song H, Fu Q, Huang K, et al. Digestion characteristics of quinoa, barley and mungbean proteins and the effects of their simulated gastrointestinal digests on CCK secretion in enteroendocrine STC-1 cells. Food Funct 2022;13:6233–6243. doi:10.1039/D2FO00243D

Sousa R, Portmann R, Dubois S, Recio I, Egger L. Protein digestion of different protein sources using the INFOGEST static digestion model. Food Res Int 2020;130:108996. doi:10.1016/j.foodres.2020.108996

Stein HH, Sève B, Fuller MF, Moughan PJ, de Lange CFM. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. J Anim Sci 2007;85:172–180. doi:10.2527/jas.2005-742

Torcello-Gómez A, Dupont D, Jardin J, et al. Human gastrointestinal conditions affect *in vitro* digestibility of peanut and bread proteins. Food Funct 2020;11:6921–6932. doi:10.1039/D0FO01451F

de Vrese M, Frik R, Roos N, Hagemeister H. Protein-Bound D-Amino Acids, and to a Lesser Extent Lysinoalanine, Decrease True Ileal Protein Digestibility in Minipigs as Determined with 15N-Labeling. J Nutr 2000;130:2026–2031. doi:10.1093/jn/130.8.2026

Wang M-F, Kishi K, Takahashi T, Komatsu T, Ohnaka M, Inoue G. Efficiency of utilization of soy protein isolate in Japanese young men. J Nutr Sci Vitaminol (Tokyo) 1983;29:201–216. doi:10.3177/jnsv.29.201

Yang A, Yu X, Zheng A, James AT. Rebalance between 7S and 11S globulins in soybean seeds of differing protein content and 11SA4. Food Chem 2016;210:148–155. doi:10.1016/j.foodchem.2016.04.095

Yuanqing H, Min C, Lingling S, et al. Ultrasound Pretreatment Increases the Bioavailability of Dietary Proteins by Dissociating Protein Structure and Composition. Food Biophys 2020; 15:409–415. doi:10.1007/s11483-020-09634-y